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TITLE: The Role of Serotonin in Hot Flashes after Breast Cancer

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14. ABSTRACT Hot flashes are a frequent, severe and bothersome symptom for women with breast cancer. Unfortunately, this symptom is difficult to treat due to limitations in understanding hot flash physiology. Although serotonin may be involved in hot flashes, it has not been directly manipulated to study effect on hot flashes. Our study purpose was to improve our understanding of the role of serotonin in hot flashes by altering central serotonin concentrations using a well-established acute tryptophan depletion paradigm. The <i>main hypotheses</i> was that alterations in central serotonin levels are involved in the induction of hot flashes in women with breast cancer and that variability in response to serotonin manipulation could be partly explained by genetic variations in the serotonin receptors and transporters. The study opened to enrollment in September, 2005 as planned with 75 women completing being our target goal. As reported in previous reports, a revised power analysis in year 2 indicated 26 participants would provide sufficient power. Overall, 28 women completed week 1 and 27 completed weeks 1 and 2. Our hypothesis was not supported. Despite achieving adequate tryptophan depletion with the active drink, we did not see any difference in objective or subjective hot flashes between the study arms. In addition, individual variation in response to depletion did not appear to be explained by genetic polymorphisms.					
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INTRODUCTION:

Among women with breast cancer, hot flashes are a frequent, severe and bothersome symptom. Unfortunately, limitations in our understanding of hot flash physiology limit clinicians' abilities to fully treat this symptom. Although current non-hormonal treatment of choice for hot flashes after breast cancer targets the central serotonin system (e.g., paroxetine, venlafaxine), the role of serotonin in hot flashes has not been directly tested. Because the efficacy of these agents has been based largely on improvement in subjective reporting of hot flashes, it is not clear whether benefits are due to physiological effects on hot flashes or due to improvements in mood or other related symptoms. In addition, these and other currently available treatments are not acceptable, appropriate, or effective for all women with breast cancer. Understanding the physiological mechanisms involved in hot flashes after breast cancer will enable us to develop more targeted behavioral and/or pharmacological therapies to be used in lieu of, or in addition to, currently available therapies so that we can eradicate hot flashes and improve the quality of life for women with breast cancer. Therefore, the *purpose of this proposal* was to improve our understanding of the role of serotonin in hot flashes by altering central serotonin concentrations using a well-established acute tryptophan depletion paradigm. The *main hypothesis* was that alterations in central serotonin levels are involved in the induction of hot flashes in women with breast cancer and that variability in response to serotonin manipulation can be partly explained by genetic variations in the serotonin receptors and transporters.

BODY:

We have completed all tasks as outlined in the approved statement of work. Below we outline the tasks outlined in the approved Statement of Work (*italics*) and indicate our accomplishments (plain text) below each.

Task 1: Develop and finalize protocol for acute tryptophan depletion, Months 1-3.

- *Meetings with investigative team, study consultants and appropriate individuals from the Indiana University General Clinical Research Center (IU GCRC) to discuss and refine experimental and control conditions.*
- *Order amino acids. Create inventory and storage protocols.*
- *Test flavor, consistency, and texture of the amino acid drinks for experimental and control conditions.*
- *Develop exact content and calorie count of mid-day snack and hi tryptophan dinner to be provided to participants during each test day.*

Protocols were developed with input from the investigative team, study consultants, and individuals from the IU GCRC laboratory services, dietary services, and nursing services. We confirmed amounts of amino acids for experimental and control conditions as original proposed in the grant application. We identified suppliers of amino acids and ordered the amino acids. All amino acids were stored at the IU GCRC dietary services department according to their standard protocols. We worked with IU GCRC dieticians to test the amino acids drinks. We determined that two flavorings were most palatable - chocolate mint and orange. We rejected a lemon flavoring as being unpalatable. We eliminated the mid-day snack per advice of our study consultant Dr. Salomon. We worked with IU GCRC services to create three dinner meal choices for participants; all with an equal amount of tryptophan. Each meal contained 0.25g of tryptophan (the amount of tryptophan in 3 ounces of cooked turkey breast).

Task 2: Prepare for enrollment of study participants, Months 1-6.

- *Finalize scripts for contacting participants, questionnaires, and other data collection forms.*

- *Create and finalize quality assurance audit forms to ensure safety of participants and integrity of all data.*
- *Generate schema for random ordering of study arms.*
- *Update IRB protocols, informed consent documents, etc.*
- *Generate standard operating procedures manual to reflect all aspects of study procedures.*
- *Refine protocols for laboratory analyses.*
- *Work with computer programmer to modify current accrual database to include scripts, screening form, etc and allow accrual and productivity reports to be generated.*
- *Work with computer programmer to create databases for entry of study data.*
- *Order supplies (hot flash monitors, electrodes, laboratory supplies, etc.).*
- *Make gel for use with hot flash monitor.*

The study team finalized scripts, a quality assurance audit form, and a randomization schema. All protocols and informed consent documents were submitted to and approved by the IRB. Standard operating procedures were generated to reflect all study activities including but not limited to the following: participant recruitment and tracking database, Biolog hot flash monitor, participant compensation, hiring and training study staff, miscellaneous office procedures (copies, computer access, etc), verifying medical records information on breast cancer disease and treatment, and scheduling / performing study visits. The laboratory analysis protocols were created and finalized by Dr. Skaar, co-investigator. The accrual database was modified to allow tracking of study accrual and productivity for day-to-day study operations. A computer programmer created a database to allow entry of study data. The database was thoroughly tested before any data entry occurred. Supplies were ordered and added to an inventory list. A supplier for the electrode gel was identified and the gel was ordered in preparation for the first patient visit.

Task 3: Subject recruitment and data collection, Months 7-30.

- *Screening and recruitment of potentially eligible women.*
- *Contact patients in existing accrual database who have agreed to be contacted for future studies.*
- *Institute procedures for recruitment of patients from breast oncology clinics.*
- *Enrollment with random ordering of experimental and control conditions.*
- *Add two medical oncologists as physician co-investigators in years 2-3 to expand number of Indiana University clinics included as recruitment sites.*
- *Make additional gel and order additional supplies (electrodes, laboratory supplies, etc.) as needed.*

Screening and recruitment of potentially eligible women was completed. We contacted patients in the existing database as planned to enroll them into the study. Recruitment in the breast oncology clinics occurred as planned. Subjects were random ordered as planned. Only one medical oncologist was added as a physician co-investigator. Other oncologists allowed recruitment at their offices without acknowledgment as co-investigator. Additional gel and supplies was ordered as needed to complete participant visits.

A total of 260 women were approached and 39 were not interested in being screened for study. Of the 221 women who were screened for study, 162 were ineligible. Of the 59 eligible women, 23 were not interested leaving 36 who consented to study. Of these 36, 5 expressed lack of interest and withdrew and 3 were withdrawn due to ineligibility. Of the remaining 28 women, 28 completed week 1 and 27 completed week 2 (one withdrew after week 1 due to dizziness occurring 3 days after the study visit). Genetic analyses were available for all women. However,

due to difficulties in sustaining venous access, only 24 women had serial tryptophan and metabolites data during the depletion week and only 23 during the control week.

The 27 subjects who completed both study arms were mostly Caucasian (93%), married or living with a partner (82%), and with household incomes above \$60K (59%). Mean age was 53.4 years old (SD= 9.6, range 30-71). All had been successfully treated for non-metastatic breast cancer (e.g., stage III or less) and were considered to be free of cancer at the time of the study. Mean time post-completion of primary treatment (e.g., surgery, chemotherapy, radiation) was 37.8 months (SD=18.6, range 13-77). Breast cancer treatments received included surgery alone (15%), surgery with radiation therapy (15%), surgery with chemotherapy (19%), and surgery with radiation and chemotherapy (52%). No subjects were taking hormone replacement therapy, 37% were taking tamoxifen, 42% were taking an aromatase inhibitor, and 33% were taking a selective serotonin reuptake inhibitor or selective serotonin and norepinephrine reuptake inhibitor (SSRI/SNRI). No significant differences were found between those randomized to depletion/control versus control/depletion for age, race, marital status, education, employment, income, or SSRI/SNRI use ($p > .10$).

Task 4: Ongoing quality assurance audits to ensure patient safety and data integrity, Months 7-30.

- *Twice monthly monitoring of activities (number of screening phone calls logged, number and type of contacts with potential or actual participants, progress with data entry, etc).*
- *Twice monthly monitoring of study accrual, including accrual of minority women.*
- *Continuous monitoring/reporting of potential adverse events.*
- *Monthly audits to verify study staff adherence to standard operating procedures. Retrain staff as needed.*
- *Monitoring of inter-rater reliability for scoring hot flash monitor data (RA and Dr. Carpenter) and Hamilton depression scale scoring (Drs. Johns, Salomon, and Carpenter).*

Throughout the study, the PI and project manager met weekly to twice monthly to monitor study activities and study accrual. The goal was to ensure that all potential participants were appropriately contacted, screened, and consented. Potential adverse events were monitored daily by the Project Manager. Adverse events included the following. During acute tryptophan depletion, five subjects vomited. Vomiting occurred within 20 minutes after ingesting amino acids ($n=1$) or between 4 and 6.25 hours later ($n=4$). One patient experienced hypoglycemia 3 hours after drink ingestion both study visits that was resolved with glucose tablets. Mood was not significantly impacted. Hamilton Rating Scale-Depression scores remained < 18 at all time points and for both study arms. Similarly, total mood disturbance and symptom rating scores were not significantly different between study arms over time ($p > .20$). The project manager reviewed adherence to standard operating procedures on a regular basis (monthly to weekly). IU GCRC staff and research assistants were retrained as necessary. Inter-rater reliability for hot flash monitor data and Hamilton depression scale scoring was done every 6 months and always exceeded 90% for both measures.

Task 5: Interim analyses, Months 12 and 24

- *Interim statistical analysis.*
- *Preparation and submission of abstracts reflecting findings to date.*
- *Creation and submission of annual reports to funding agency.*

We performed the interim analysis as outlined in our year 1 and year 2 reports. In the first analysis, we performed a case study analysis. Results for the first four participants suggested that 3 of 4 participants responded *the opposite of what we hypothesized*. In other words, the

breast cancer survivors are trending to have fewer hot flashes during the acute tryptophan depletion when central serotonin is lowest. Our first study participant remarked that she "felt like her internal thermostat had been reset" during the acute tryptophan depletion day. Due to the small sample, these results are not yet statistically significant (non-parametric Wilcoxon-Signed Ranks test of differences, $p = 0.45$). Because of these findings, we increased the frequency of blood draws to include hourly blood draws to closely monitor the response to the depletion and control conditions. In the second analysis, we completed an interim power analysis using PASS software. The purpose was to estimate a sample size from approximately 20 to 30 patients with at least 80% power and $\alpha=0.05$. The design was based on a 1 sample t-test of the difference between number of hot flashes on treatment and control since a Poisson distribution of counts is assumed to be approximately normal for the planned sample size. Findings indicated that 25 patients would provide 80% power to detect a significant difference between tryptophan depletion and control. We also prepared and submitted abstracts for submission (see presentations section under Reportable Outcomes). We created and submitted the annual reports as required.

Task 6: Final analyses and dissemination, Months 30-36.

- *Final statistical analyses.*
- *Preparation and submission of final report to funding agency.*
- *Preparation and submission of abstracts and manuscripts reflecting final results.*

The final analysis was completed and a manuscript was submitted and accepted for publication. The final funding agency report is this report here. Additional abstracts were submitted (see Presentations section under Reportable Outcomes). Results of the final statistical analysis are below (see Key Research Accomplishments) and in the attached appendix.

KEY RESEARCH ACCOMPLISHMENTS:

Bulleted list of key findings derived under the award as requested in the March 2008 feedback document:

- Adequate separation between study arms was achieved between hours 3 and 8 of the protocol. Baseline, 1 hour, and 2 hour total tryptophan values were not significantly different ($p=.46$) between the group randomized to depletion treatment first and the group randomized to the depletion second. 3, 4, 5, 6, 7, 8 hour total tryptophan values were significantly lower for the tryptophan depletion group compared to the control group. As expected, during the nadir period (5 hours after drink ingestion), 83% of women receiving tryptophan depletion had total tryptophan values $< 10 \mu\text{M}$ compared to 35% while on control ($p = 0.001$). Similarly, nadir TRP-LNAA ratios were significantly lower at hour 5 during tryptophan depletion ($M=0.0034$, $SD=0.0027$) compared to control ($M=0.02$, $SD=0.02$) ($p=< 0.0001$).
- Contrary to our hypothesis, there were no statistically significant differences in any hot flash variables (subjective hot flash frequency, intensity, bother, objective hot flash frequency, skin conductance magnitude) between depletion and control conditions for any of the 8-hour long variables or any of the 3-hour long tryptophan nadir variables. Also not significant were differences between groups for 5-hour (nadir) tryptophan response and TRP/LNAA response. Response to the acute tryptophan depletion did not vary by use of SSRI/SNRI, anti-estrogens, breast cancer disease and treatment variables.
- Also contrary to our hypothesis, response to acute tryptophan depletion did not vary by genetic variants in the HTR1A, HTR2A, and TRH1 genes

Bulleted list of novel research findings as requested in the March 2009 feedback document (received via email in February, 2010):

- This was the first study to evaluate menopausal hot flashes in relation to tryptophan depletion.
- There were no statistically significant differences in any hot flash variables (subjective hot flash frequency, intensity, bother, objective hot flash frequency, skin conductance magnitude) between depletion and control conditions.
- Response to the acute tryptophan depletion did not vary by use of SSRI/SNRI, anti-estrogens, breast cancer disease and treatment variables.
- Response to acute tryptophan depletion did not vary by genetic variants in the HTR1A, HTR2A, and TRH1 genes.

Addition of data presentation in the body of the report and citation of data in the appendices to support key research findings as requested in email accompanying March 2009 feedback document (received via email in February 2010):

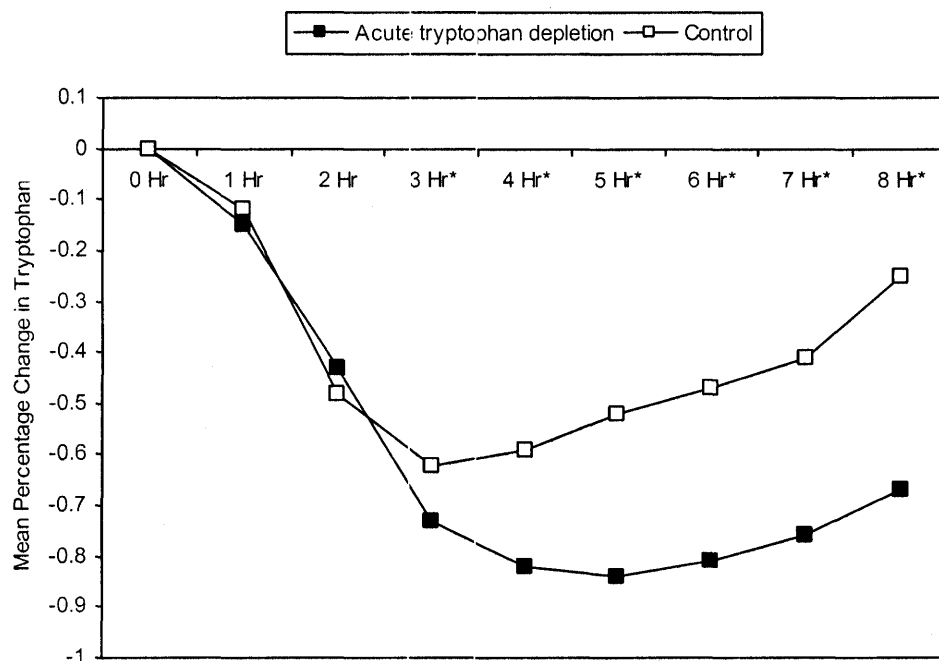
Below is a description of study results (please refer to appended published manuscript). Of the 36 consenting women, 5 expressed lack of interest and withdrew and 3 were withdrawn due to ineligibility. Of the remaining 28 women, 28 completed week 1 and 27 completed week 2 (one withdrew after week 1 due to dizziness occurring 3 days after the study visit). Genetic analyses were available for all women. However, due to difficulties in sustaining venous access, only 24 women had serial tryptophan and metabolites data during the depletion week and only 23 during the control week.

The 27 subjects were mostly Caucasian (93%), married or living with a partner (82%), and with household incomes above \$60K (59%). Mean age was 53.4 years old (SD= 9.6, range 30-71). All had been successfully treated for non-metastatic breast cancer (e.g., stage III or less) and were considered to be free of cancer at the time of the study. Mean time post-completion of primary treatment (e.g., surgery, chemotherapy, radiation) was 37.8 months (SD=18.6, range 13-77). Breast cancer treatments received included surgery alone (15%), surgery with radiation therapy (15%), surgery with chemotherapy (19%), and surgery with radiation and chemotherapy (52%). No subjects were taking hormone replacement therapy, 37% were taking tamoxifen, 42% were taking an aromatase inhibitor, and 33% were taking an SSRI/SNRI. No significant differences were found between those randomized to depletion/control versus control/depletion for age, race, marital status, education, employment, income, or SSRI/SNRI use ($p > .10$). Adverse events included the following. During acute tryptophan depletion, five subjects vomited. Vomiting occurred within 20 minutes after ingesting amino acids ($n=1$) or between 4 and 6.25 hours later ($n=4$). One patient experienced hypoglycemia 3 hours after drink ingestion both study visits that was resolved with glucose tablets. Mood was not significantly impacted. Hamilton Rating Scale-Depression scores remained < 18 at all time points and for both study arms. Similarly, total mood disturbance and symptom rating scores were not significantly different between study arms over time ($p > .20$).

The percentage change in serum tryptophan during depletion and control conditions is shown in Figure. Baseline, 1 hour, and 2 hour total tryptophan values were not significantly different ($p=.46$) between the group randomized to depletion treatment first and the group randomized to the depletion second. However, the tryptophan depletion group had significantly lower total tryptophan than control at hours 3 through 8 indicating that adequate separation between study arms was achieved. As expected, during the nadir period (5 hours after drink ingestion), 83% of women receiving tryptophan depletion had total tryptophan values $< 10 \mu\text{M}$ compared to 35% while on control ($p = 0.001$). Similarly, nadir TRP-LNAA ratios were significantly lower at hour 5

during tryptophan depletion ($M=0.0034$, $SD=0.0027$) compared to control ($M=0.02$, $SD=0.02$) ($p < 0.0001$).

Figure 3: Percentage Change in Serum Tryptophan Over Time During Acute Tryptophan Depletion (ATD) Versus Control Arms



Legend: This figure denotes the percentage change in tryptophan from baseline over time for each study arm ($n=27$). Times 0 to 8 refer to hourly blood draws during each test day. The solid square refers to the active amino acid drink and the open square to the control drink. Repeated measures analysis indicated that tryptophan values were not significantly different ($p=.46$) between randomized groups at hours 0, 1, or 2 but were significantly lower in the acute tryptophan depletion group compared to control group at hour 3 ($p < .01$) and hours 4, 5, 6, 7, and 8 ($p < .001$). Significant differences are shown with an asterisk next to the time point (i.e. hours 3 through 8).

As shown in Table 1, objective hot flash frequency and magnitude were highly correlated. Similarly, subjective hot flash variables (diary frequency, event button frequency, intensity, bother) were highly correlated. Correlations between objective and subjective hot flash variables were modest ($.46 < r < .58$) in this study, but higher than correlations for similar data collected during ambulatory monitoring outside of a laboratory ($.06 < r < .26$).⁶⁰

Table 1: Pearson Correlations Denoting Relationships Among Hot Flash Variables

	1	2	3	4	5	6
1. Objective frequency	---	.96***	.68**	.71**	.69**	.64**
2. Skin conductance magnitude	.97***	---	.69**	.73***	.69**	.61**
3. Subjective frequency (diary)	.42*	.48*	---	.94***	.80***	.79***
4. Subjective frequency (event button)	.43*	.49*	.98***	---	.85***	.84***
5. Subjective intensity	.43*	.46*	.83***	.77***	---	.94***
6. Subjective bother	.32	.35	.77***	.74***	.89***	---

Table note: The numbers in columns 2 to 7 correspond with the numbered variables listed in column 1, such that 1 is objective frequency, 2 is skin conductance magnitude, etc. Correlations above the diagonal are total or mean values during entire 8 hour acute tryptophan depletion condition. Correlations below the diagonal are total or mean values during entire 8 hour control condition. * $p < .05$, ** $p < .01$, *** $p < .001$

Table 2 shows no significant differences in any hot flash variables between depletion and control conditions for any of the 8-hour long variables or any of the 3-hour long tryptophan nadir variables.

Table 2: Descriptive Statistics for Hot Flash Variables During Acute Tryptophan Depletion (ATD) and Control Arm for 8 Hour Test Day and 3 Hour Nadir Periods

	8 Hour Test Day ^a			3 Hour Nadir Period ^b		
	ATD M(SD)	Control M(SD)	p	ATD M(SD)	Control M(SD)	p
Objective frequency	2.30 (2.92)	2.62 (3.29)	.71	1.22 (1.69)	1.23 (1.66)	.99
Skin conductance magnitude ^c	8.54 (9.17)	9.20 (11.24)	.82	4.29 (4.92)	4.36 (5.54)	.96
Subjective frequency (diary)	4.56 (3.11)	3.65 (3.02)	.29	1.85 (1.66)	1.42 (1.39)	.31
Subjective frequency (event button)	4.41 (2.93)	3.54 (3.11)	.30	1.85 (1.49)	1.38 (1.55)	.27
Subjective intensity	16.81 (15.87)	13.69 (16.30)	.48	7.22 (7.83)	5.81 (7.30)	.50
Subjective bother	14.26 (15.29)	10.23 (12.73)	.30	6.33 (7.64)	4.65 (6.41)	.39

^a From drink ingestion to 8 hours later.

^b Three hour period spanning 5 to 8 hours after drink ingestion.

^c Sum of all changes in skin conductance that accompanied objective and subjective hot flashes.

Not shown are t-tests for differences between groups based on tryptophan response and TRP/LNAA response. These t-tests were similar and non-significant. In addition, response did not vary by use of SSRI/SNRI, anti-estrogens, breast cancer disease and treatment variables, or genetic variants in the HTR1A, HTR2A, and TRH1 genes.

REPORTABLE OUTCOMES:

Manuscripts:

- Carpenter, J. S., Yu, M., Wu, J., Von Ah, D., Milata, J., Otte, J. L., Johns, S., Schneider, B., Storniolo, A. M., Salomon, R., Desta, Z., Cao, J., Jin, Y., Philips, S., & Skaar, T. (in press). Evaluating the role of serotonin in hot flashes after breast cancer using acute tryptophan depletion. *Menopause*.

Presentations – ***abstract copies in appendices as requested in the March 2009 feedback document (received via email in February, 2010):**

National / International presentations:

- *Carpenter, J.S. Skaar, T., Storniolo, A. M., Milata, J., Wu, J., & Yu, M. (Oct, 2008). Acute tryptophan depletion, central serotonin, and hot flashes after breast cancer. 2008 National State of the Science Congress in Nursing Research (Council for Advancement of Nursing Science), Washington, DC.
- *Carpenter, J. S., Skaar, T., Storniolo, A. M., Jin, Y., Desta, Z., & Johns, S. (Feb, 2007). The role of serotonin in hot flashes after breast cancer. 9th National Conference on Cancer Nursing Research, Hollywood, CA.

- *Carpenter, J., Skaar T., Storniolo, A.M., Jin, Y., Desta, Z. & Johns, S. (July, 2006). The role of serotonin in hot flashes after breast cancer. Collegium Internationale Neuropsychopharmacologicum 29th Annual Congress, Chicago, IL.

Regional / Local Presentations:

- Carpenter, J.S. (May, 2007) The Hot Flash, Presenter for the 2006-07 Women's Health Noon Lecture Series at the IU Cancer Research Institute Auditorium, Indianapolis, IN.
- Carpenter, J. S. (Aug, 2006). Hot flashes after breast cancer, Indianapolis Breast Center Staff Inservice, Indianapolis, IN, August 16th.
- *Carpenter, J., Johns, S., Storniolo, A.M., Jin, Y., Desta, Z. & Skaar T. (Feb, 2006). Update on the Role of Serotonin in Hot Flashes after Breast Cancer. The Amelia Project: Giving Wings to Research, Indianapolis, IN.
- *Carpenter, J., Johns, S., Monahan, P., Storniolo, A.M., Jin, Y., Desta, Z. & Skaar T. (Feb, 2005). The Role of Serotonin in Hot Flashes after Breast Cancer. The Amelia Project: Giving Wings to Research, Indianapolis, IN.

Correlative studies:

One correlative study was conducted during the award period with additional funding. The lead investigator on the correlative study was postdoctoral fellow Diane Von Ah, PhD, RN with co-mentors Janet S. Carpenter, PhD, RN and Todd Skaar, PhD. Dr. Von Ah successfully evaluated the effect of acute tryptophan depletion on memory and neuropsychological functioning in breast cancer survivors. The correlative study was funded through two training grants:

- Genetics Fellowship to Enhance Oncology Symptom Management (Carpenter & Skaar co-mentors; D. Von Ah Post Doctoral Fellow), ONSF RE02 \$18,700 total costs. 09/01/05 - 08/31/06
- Serotonin and Cognitive Dysfunction in Breast Cancer (Carpenter & Skaar co-mentors; D. Von Ah Post Doctoral Fellow, Indiana University Clinical Research Feasibility Funds Program (CReFF) \$20,000 total costs. 03/01/06 – 02/28/07.

Dr. Von Ah received the Victoria Mock New Investigator Award from the Oncology Nursing Society (2009) for her work in cognitive dysfunction. As a recipient she was invited to present her findings: ***abstract copies in appendices as requested in the March 2009 feedback document (received via email in February, 2010):**

- *Von Ah, D., Skaar, R., Unverzagt, F., Yu, M., Wu, J., Moser, L., Ryker, K., Milata, J., & Carpenter, J.S. (Feb, 2009). Cognitive dysfunction in breast cancer survivors: The role of serotonin. 10th National Conference on Cancer Nursing Research, Orlando, Florida.

Study results from the correlative study are being prepared in manuscript format for summer, 2009 submission:

- Von Ah, D., Skaar, R., Unverzagt, F., Yu, M., Wu, J., Schneider, B., Storniolo, A. M., Moser, L., Ryker, K., Milata, J., & Carpenter, J.S. (In progress 2009). Cognitive dysfunction in breast cancer survivors: The role of serotonin. To be submitted to *Journal of Psychopharmacology*.

Results from the correlative study were presented nationally and locally: ***abstract copies in appendices as requested in the March 2009 feedback document (received via email in February, 2010):**

- *Von Ah, D., Carpenter, J. S., Unverzagt, F., & Skaar, T. (Mar, 2008). "Cognitive Dysfunction in Breast Cancer Survivors: The Role of Serotonin," 2008 Annual Midwest Nursing Research Conference, Indianapolis, IN.
- *Von Ah, D., Carpenter, J. S., Unverzagt, F., & Skaar, T. (Feb, 2008). Cognitive dysfunction in breast cancer survivors: The role of serotonin. 10th Annual Amelia Retreat, IU Conference Center, Indianapolis, IN.

- *Von Ah, D., Carpenter, J. S., Skaar, T. (Feb, 2007). Serotonin and cognitive dysfunction in breast cancer survivors. National Conference on Cancer Nursing Research, Hollywood, CA.

Additional Research / Training Grants:

Seven additional research / training grants were submitted as a result of this project. This project helped us to form additional collaborations on campus that resulted in ideas to continue this line of research on the etiology of hot flashes. Below is a list of applications that were submitted and their outcome.

- Submitted 02/15/09: Etiology of Hot Flashes Related to Endocrine Therapies for Breast Cancer. Trainee: Sherry Pittman. Mentors: Todd Skaar, PhD and Janet S. Carpenter, PhD, RN. Clinical Translational Science Institute (CTSI) Predoctoral Training in Translational Research (\$24,000). Under review.
- Submitted 11/20/08: The role of the kynurenine metabolic pathway in the etiology of therapy induced hot flashes in breast cancer survivors. PI: Dmitry Zaretsky, PhD. Co-Is: Todd Skaar, PhD, Janet S. Carpenter, PhD, RN. DOD Concept Award (\$75,000). Under review.
- Submitted 11/20/08: The ventromedial medulla as a candidate central site for targeting therapies to relieve breast cancer therapy induced hot flashes. PI: Dmitry Zaretsky, PhD. Co-Is: Todd Skaar, PhD, Janet S. Carpenter, PhD, RN. DOD Concept Award (\$75,000). Under review.
- Submitted 07/15/08: Hot Flashes and the Disruption of Estrogen-Dependent Orexin Signaling (in Breast Cancer Patients Taking Aromatase Inhibitor). Trainee: Sherry Pittman. Mentors: Todd Skaar, PhD and Janet S. Carpenter, PhD, RN. Indiana University School of Medicine graduate fellowship in Translational Research (\$23,500). Funded.
- Submitted 06/30/08: Orexin A and Menopausal Symptoms in Breast Cancer. Co-PIs: Janet S. Carpenter, PhD, RN and Philip Johnson, PhD, Co-Is: Todd Skaar, PhD, Diane Von Ah, PhD, RN, Julie Otte, PhD, RN, and Kevin Rand, PhD. Oncology Nursing Society Foundation RE28 Breast Cancer Research Grant (\$100,000). Score=1.87 (1=outstanding, 5=very poor). Not funded.
- Submitted 02/15/08: Evaluation of comprehensive rodent model of hot flashes. PI: Dmitry Zaretsky, PhD. Co-Is: Janet S. Carpenter, PhD, RN and Todd Skaar, PhD. National Institute on Aging / National Institutes of Health R21 (\$275,000). Not scored. Not funded.
- Submitted 01/15/08: Indoleamine 2,3-dioxygenase (IDO): a novel target in a treatment of hot flushes in breast cancer survivors. PI: Dmitry Zaretsky, PhD. Co-Is: Todd Skaar, PhD. Internal Indiana University Showalter Grant. Not funded.

CONCLUSION:

The primary aim of this study was to investigate the effects of lowered serotonin synthesis by acute tryptophan depletion on hot flashes in female survivors of breast cancer - a group known to have frequent, severe, and bothersome hot flashes. It was hypothesized that acute tryptophan depletion would exacerbate hot flashes, however, our data did not support this assumption. We did not find any significant differences between study conditions for objective or subjective hot flash measures.

There are two potential explanations for these negative study findings. First, if central serotonin functioning was severely diminished in these postmenopausal women, we may not have accomplished a further reduction in serotonin with acute tryptophan depletion. Although women

are generally more susceptible than men to the effects of acute tryptophan depletion on memory,¹ estrogen loss at menopause may affect how postmenopausal women respond to acute tryptophan depletion. Loss of estrogen appears to diminish serotonin concentrations, activity and metabolism²⁻⁶ Thus, we may not have accomplished further lowering of central serotonin despite the observed changes in peripheral tryptophan and TRP/LNAA ratios. This would need to be tested in a subsequent study using cerebral spinal fluid sampling. An alternative method to test our hypothesis that central serotonin is involved in the induction of hot flashes would be to administer tryptophan and assess whether hot flashes are alleviated. Tryptophan supplements have been suggested as a potential hot flash treatment⁷ and appear to impact other symptoms. For example, in women, tryptophan significantly reduced premenstrual dysphoric symptoms compared to placebo.^{8,9} In humans, primates, and rats, tryptophan decreases mild depressive symptoms and aggressive behavior.^{10,11} Chronic tryptophan supplementation raises plasma tryptophan and central serotonin and potentiates SSRI effectiveness in rats.^{12,13} Although contaminated tryptophan supplements were thought to cause eosinophilia myalgia syndrome,¹⁴ today, dietary manipulation of tryptophan or L-tryptophan/5-hydroxytryptophan supplementation is a safe intervention.^{10,11,13,15}

A second explanation for negative study findings may be that acute tryptophan depletion alone, without an additional stressor such as a thermal challenge, may not have been adequate to invoke hot flashes. A similar paradigm has been seen in panic and obsessive compulsive disorders. Tryptophan depletion alone does not provoke panic, however, it increases susceptibility to panic so that if a stressor is applied during tryptophan depletion (i.e., flumazenil), panic symptoms are exacerbated.¹⁶ Similarly, symptoms of obsessive compulsive disorder emerge after acute tryptophan depletion only when an additional stressor is present.¹⁷ In this study, tryptophan depletion may have increased susceptibility to hot flashes but normal environmental stressors were controlled due to the laboratory setting (e.g., absence of temperature and humidity fluctuations, absence of emotional stressors). This hypothesis could be tested in a follow-up study by comparing time to hot flash onset or hot flash frequency, intensity or bother following a stressor such as application of heating pads during acute tryptophan depletion and control conditions.

Study findings should be considered in light of study limitations. Although the sample size was comparable or larger than that seen in over 70 similar studies included in a recent review,¹⁸ the sample was limited in terms of ethnic and racial diversity, yet heterogeneous in relation to breast cancer disease and treatment characteristics. In addition, this sample of breast cancer survivors does not provide any indication of how healthy menopausal women who have not undergone breast cancer treatment would respond. Finally, the design was limited in that it did not include a study arm to evaluate the effects of tryptophan or 5-hydroxytryptophan supplementation. Some previous studies have used tryptophan supplementation for comparison.¹⁶ However, because of reported variable increases in tryptophan and serotonin with supplementation¹⁹ and anticipated difficulties in recruiting women to take part in an additional study visit, we chose to use a one-quarter strength drink as the control condition. This control condition reduced plasma tryptophan levels more than the 25% that was expected and the reasons for this are unknown. However, the control drink did differ from the full strength depletion in that depletion occurred to a lesser degree and with faster recovery.¹⁹

In summary, to our knowledge, this was the first study to examine hot flash physiology using acute tryptophan depletion. Further testing of our study hypothesis may be warranted through tryptophan supplementation or a tryptophan depletion with stressor paradigm. Findings indicate that additional research on mechanistic pathways is needed to guide appropriate treatment in clinical practice.

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APPENDICES: Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

Appendix 1 - **published** manuscript

Appendix 2 – copies of abstracts from listed presentations

SUPPORTING DATA:

Supporting data (statistical analysis, results, discussion, figures, tables) are included in the published manuscript (see appendix). In addition, included below are two tables that were not part of the final manuscript. The first table compares hot flash variables between subjects who were classified as good and poor responders to the acute tryptophan depletion paradigm. Good responders were first defined as subjects whose tryptophan concentrations were $< 10 \mu\text{M}$ at the nadir point (5 hours after drink ingestion). Table A below shows no difference in hot flash variables based on good or poor response to the depletion when comparing 8-hour test day hot flashes and the subset of 3-hour nadir hot flashes.

Table A: Descriptive Statistics for Hot Flash Variables based on TRP responder for 8 Hour Test and 3 Hour Nadir Periods

	8 Hour Test Period ^a		p-value	3 Hour Nadir Period ^b		p-value
	Good M(SD)	Poor M(SD)		Good M(SD)	Poor M(SD)	
Objective hot flash frequency	2.79 (2.95)	2.63 (3.53)	0.8718	1.43 (1.71)	1.32 (1.73)	0.8263
Skin conductance magnitude ^c	9.61 (9.38)	9.91 (12.07)	0.9268	4.81 (4.87)	4.84 (6.00)	0.9853
Subjective hot flash frequency (diary)	4.57 (3.06)	4.32 (3.07)	0.7804	1.93 (1.65)	1.63 (1.34)	0.5189
Subjective hot flash frequency (event marker)	4.46 (2.92)	4.05 (3.21)	0.6509	1.96 (1.57)	1.47 (1.47)	0.2872
Subjective hot flash intensity	16.89 (16.23)	15.95 (16.75)	0.8474	7.46 (8.16)	6.47 (6.92)	0.6667
Subjective hot flash bother	14.96 (16.10)	11.53 (11.73)	0.4296	6.96 (8.09)	5.00 (5.73)	0.3658

^a From drink ingestion to 8 hours later. No significant differences between ATD and control during 8 hour test day.

^b From 5 to 8 hours after drink ingestion. No significant differences between ATD and control during 3 hour nadir.

^c Sum of all changes in skin conductance that accompanied objective and subjective hot flashes.

We then classified good responders as those who achieved tryptophan/large neutral amino acid (TRP/LNAA) ratios as $< .007$ and compared those women to poor responders (e.g., TRP/LNAA ratio $\geq .007$). Again, no differences in hot flash variables were seen between those whose tryptophan and serotonin was most severely depleted vs. those who were less depleted. Table B shows shows no difference in hot flash variables based on good or poor response to the depletion using TRP/LNAA ratios when comparing 8-hour test day hot flashes and the subset of 3-hour nadir hot flashes.

Table B: Descriptive Statistics for Hot Flash Variables based on TRP/LNAA Ratio responder for 8 Hour Test and 3 Hour Nadir Periods

	8 Hour Test Period ^a		p-value	3 Hour Nadir Period ^b		p-value
	Good M(SD)	Poor M(SD)		Good M(SD)	Poor M(SD)	
Objective hot flash frequency	2.72 (3.06)	2.73 (3.34)	0.9938	1.44 (1.69)	1.32 (1.76)	0.8095
Skin conductance magnitude ^c	9.73 (9.81)	9.75 (11.32)	0.9945	4.86 (4.99)	4.78 (5.74)	0.9585
Subjective hot flash frequency (diary)	4.68 (3.11)	4.23 (3.01)	0.6152	2.00 (1.68)	1.59 (1.33)	0.3652
Subjective hot flash frequency (event marker)	4.48 (2.95)	4.09 (3.15)	0.6637	2.00 (1.58)	1.50 (1.47)	0.2699
Subjective hot flash intensity	17.36 (16.02)	15.55 (16.87)	0.7072	7.60 (7.79)	6.45 (7.56)	0.6125
Subjective hot flash bother	14.96 (15.61)	12.00 (13.21)	0.4896	7.00 (7.64)	5.23 (6.76)	0.4070

^a From drink ingestion to 8 hours later, No significant differences between ATD and control during 8 hour test day.

^b From 5 to 8 hours after drink ingestion. No significant differences between ATD and control during 3 hour nadir.

^c Sum of all changes in skin conductance that accompanied objective and subjective hot flashes. Note: A similar pattern of results was seen good responders were compared to poor responders. Good responders were women whose tryptophan or tryptophan/LNAA ratios were as expected during depletion. Poor responders were women whose tryptophan or tryptophan/LNAA ratios were as expected during depletion

Appendix 1

Published manuscript

Evaluating the role of serotonin in hot flashes after breast cancer using acute tryptophan depletion

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Abstract

Objective: Among women with breast cancer, hot flashes are frequent, severe, and bothersome symptoms that can negatively impact quality of life and compromise compliance with life-saving medications (eg, tamoxifen and aromatase inhibitors). Clinicians' abilities to treat hot flashes are limited due to inadequate understanding of physiological mechanisms involved in hot flashes. Using an acute tryptophan depletion paradigm, we tested whether alterations in central serotonin levels were involved in the induction of hot flashes in women with breast cancer.

Methods: This was a within-participant, double-blind, controlled, balanced, crossover study. Twenty-seven women completed two 9-hour test days. On one test day, women ingested a concentrated amino acid drink and encapsulated amino acids (no tryptophan) according to published procedures that have been shown to have specific effects on serotonin within 4.5 to 7 hours. On the other test day, women ingested a control drink. Serial venous blood sampling and objective hot flash monitoring were used to evaluate response to each condition.

Results: Response to acute tryptophan depletion was variable and unexplained by use of selective serotonin reuptake inhibitors, antiestrogens, breast cancer disease and treatment variables, or genetic polymorphisms in serotonin receptor and transporter genes. Contrary to our hypothesis, hot flashes were not worsened with acute tryptophan depletion.

Conclusions: Physiologically documented and self-reported hot flashes were not exacerbated by tryptophan depletion. Additional mechanistic research is needed to better understand the etiology of hot flashes.

Key Words: Hot flashes – Menopause – Tryptophan – Serotonin – Breast cancer.

For breast cancer survivors, the hot flash is a frequent, severe, bothersome, and persistent problem¹⁻⁵ that negatively impacts daily activities, mood, sleep, and overall quality of life.⁶ Abrupt withdrawal of hormone therapy at the time of breast cancer diagnosis and treatment with selective estrogen receptor modulators,⁷⁻¹⁰ aromatase inhibitors,¹¹ or chemotherapy^{10,12} can precipitate and/or exacerbate hot flashes. Treating hot flashes in this group is difficult due to contraindications against hormone therapy^{13,14} and limited understanding of hot flash etiology.

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Although there is strong evidence implicating reduced serotonin in the etiology of hot flashes,^{15,16} the mechanism underlying serotonin involvement is unclear. Low serotonin has been observed in women after spontaneous or surgical menopause.^{17,18} Estrogen replacement alleviates hot flashes and restores serotonin concentrations.^{17,18} Estradiol also augments serotonergic activity in postmenopausal women.¹⁹ Estrogen seems to affect serotonin metabolism through direct effects on serotonin neurons, which regulate genes involved in serotonin synthesis, transport, and signaling.^{20,21} Patients with carcinoid tumors also experience hot flashes.^{22,23} Although carcinoid tumors are associated with high peripheral serotonin levels, presumably, central serotonin levels are low.^{22,23} Similarly, elevated peripheral serotonin concentrations in perimenopausal women that were positively correlated with hot flashes likely reflect low central levels of serotonin.²⁴ Based on these and other data, selective serotonin reuptake inhibitors (SSRIs) or selective norepinephrine reuptake inhibitors (SNRIs) are frequently used to treat hot flashes.^{25,26}

One method for evaluating the role of central serotonin in hot flashes is the acute tryptophan depletion paradigm. This paradigm has been widely used and accepted within the field of psychiatry to evaluate the role of central serotonin

neurotransmission in various disorders such as depression, panic disorder, and premenstrual syndrome.²⁷⁻³⁰ It can be used without serious medical or psychological complications even in women with such disorders.^{28,30-33} This paradigm alters serotonin function in humans, as confirmed with cerebrospinal fluid sampling,³⁴ without the side effects associated with pharmacological agents. In addition, effects are specific for serotonin. There are no direct effects on other neurotransmitters.³²

Tryptophan is the precursor for serotonin synthesis and is a naturally occurring amino acid found in foods such as turkey, cheese, and nuts. Because tryptophan is transported into the brain and can be rate limiting for serotonin synthesis, decreasing circulating tryptophan causes a temporary suppression of central serotonin synthesis and concentrations. Acute tryptophan depletion is accomplished by administering a 80- to 100-g amino acid drink that contains no tryptophan. Central tryptophan becomes temporarily depleted as (1) hepatic protein synthesis uses up the existing tryptophan and (2) tryptophan competes at a relative disadvantage with the other large neutral amino acids supplied in the drink for the amino acid transporters that transport tryptophan into the brain.^{31,32} Acute tryptophan depletion results in temporary lowering of central serotonin neurotransmission within 5 to 7 hours.^{28,32} Adverse effects (nausea, mood changes) are mild and subside after ingestion of a tryptophan-containing meal.

The purpose of this study was to directly reduce central serotonin via acute tryptophan depletion and study acute effects on hot flashes in breast cancer survivors. Our main hypothesis was that deficits in central serotonin levels were involved in the induction of hot flashes in these women who are at heightened vulnerability to hot flashes due to their cancer treatments. Because genetic variations in serotonin receptor and transporter genes have been documented,³⁵⁻³⁸ we also hypothesized that variability in response to serotonin manipulation could be partly explained by genetic variations.

METHODS

Sample and setting

Participants were recruited from a Midwestern outpatient cancer clinic from 2005 to 2007. All procedures were approved by a local institutional review board, a cancer center scientific review committee, and the funding agency human subjects review board. All participants were (1) adults at least 18 years of age (actual age range, 30-71 y); (2) willing and able to provide informed consent; (3) reporting daily hot flashes; (4) able to read, write, and speak English; (5) postmenopausal (≥ 12 -mo amenorrhea^{39,40}); and (6) greater than 1 month but less than 5 years posttreatment (surgery, radiation, chemotherapy) for nonmetastatic breast cancer. These criteria allowed inclusion of women successfully treated for recurrent breast cancer because there was no known reason to exclude them.¹⁰ Exclusion criteria were as follows: (1) current depression, (2) history of migraines, (3) history of hepatitis, and (4) abnormal chemistry profile (eg, sodium, potassium, and glucose). Women taking SSRIs/SNRIs were not excluded

for two reasons: (1) acute tryptophan depletion was expected to produce a relapse of hot flash symptoms in SSRI/SNRI users,^{31,32} and (2) we did not want to bias the sample toward those who may not be taking SSRIs/SNRIs for hot flashes due to lack of efficacy.

Design and intervention

The study was a within-participant, double-blind, controlled, balanced, crossover trial. Participants took part in two 9-hour clinic visits 7 days apart. They received in random order either acute tryptophan depletion drink and capsules or a one-quarter strength control drink and capsules.

Acute tryptophan depletion was achieved with a 300-mL amino acid drink and encapsulated amino acids containing 100 g total amino acids in the following ratio: L-alanine (5.5 g), L-arginine (4.9 g), L-cysteine (2.7 g), glycine (3.2 g), L-histidine (3.2 g), L-isoleucine (8.0 g), L-leucine (13.5 g), L-lysine (11.0 g), L-methionine (3.0 g), L-phenylalanine (5.7 g), L-proline (12.2 g), L-serine (6.9 g), L-threonine (6.9 g), L-tyrosine (6.9 g), and L-valine (8.9 g).⁴¹⁻⁴³ Orange or chocolate mint flavoring was used to improve palatability of the drink. Cysteine, methionine, and arginine were encapsulated due to their unpleasant taste.^{34,41,43} We used a 100-g drink rather than the 80-g drink used in some studies to produce the greatest effect. The 100-g drink was expected to produce acute tryptophan depletion within 4.5 to 7 hours, with a 80% to 90% drop in plasma tryptophan^{31,33,34} and concomitant drop in central tryptophan.³⁴

The control arm was a 300-mL drink and encapsulated amino acids but in one-fourth strength: L-alanine (1.4 g), L-arginine (1.2 g), L-cysteine (0.7 g), glycine (0.8 g), L-histidine (0.8 g), L-isoleucine (2.0 g), L-leucine (3.4 g), L-lysine (2.8 g), L-methionine (0.8 g), L-phenylalanine (1.4 g), L-proline (3.1 g), L-serine (1.7 g), L-threonine (1.7 g), L-tyrosine (1.7 g), L-valine (2.2 g), and fillers (7.95 g).^{32,44} The control drink was expected to lower tryptophan only 25%. Other studies have used tryptophan supplementation as a control condition; however, this practice has been criticized because changes in tryptophan and serotonin can be highly variable.³² We did not compare depletion to both the control and supplementation because this would have required participants to take part in three all-day study visits.

Randomization and blinding

Biostatisticians created a computer-generated randomization sequence, with randomization done in blocks of four without stratification. After informed consent was obtained, the participant's study number was sent to the study dietitian who randomized the participant to one of two sequence groups: acute tryptophan depletion/control or control/acute tryptophan depletion. The study dietitian carried out the randomization using amino acids supplied by the investigative team. The dietitian dispensed depletion and control drinks in metal cups with black opaque lids and straws to prevent participants, nurses, or study staff from seeing the drink contents. The project manager checked each drink outside the participant's room to ensure that the dietitian had carried out

the randomization appropriately. Thus, the dietitian and the project manager were not blinded. Participants and specified team members (nurses, data collectors, hot flash analysts, data entry personnel, laboratory technicians) were blinded. In the consent form, participants were told that they would receive two different amino acid drinks in random order. The notion of an active drink and a control or placebo drink was never introduced to them. Thus, participants were not aware that an active drink was being compared with a control drink, and participants who noticed a difference in the viscosity of the drinks were not able to link the more viscous drink to the active condition. In addition, the number of capsules for the unpleasant tasting amino acids was identical between arms.

Study procedures

Women were recruited in the cancer clinic or by telephone after being referred to the study by clinic staff. They were told about the study, were screened for eligibility, and, if eligible and interested, were mailed a packet of study materials and asked to return signed consent forms in prepaid envelopes. After consenting, women were interviewed by the study psychologist who verified their eligibility as non-depressed using the Hamilton Rating Scale-Depression. Women were then scheduled for their first study visit.

Procedures for the two study weeks were identical. Participants arrived at the General Clinical Research Center after fasting for 8 hours. Outpatient admission procedures included the following: obtaining a brief medical history, physical examination, vital signs, height, and weight; collecting a urine sample for drug screening; placing an intravenous catheter with heparinized saline for blood draws; and starting objective hot flash monitoring. To obtain a baseline, blood was drawn and the participant was asked to fill out demographic, symptom, and mood questionnaires. The study start (time 0) coincided with ingestion of the amino acids. Subsequent blood draws were completed hourly for 8 hours. Symptom and mood questionnaires were completed 3, 5, and 7 hours later. Participants engaged in quiet activities while being monitored by nurses. Eight hours later, a meal containing 0.25 g of tryptophan was served (the amount of tryptophan in 3 oz of cooked turkey breast). The study psychologist verified absence of depressive symptoms, the intravenous access was removed, and women were discharged wearing a hot flash monitor. The next morning, a trained nurse telephoned participants with instructions for turning off and disconnecting the hot flash monitor. The nurse verified the absence of depression using the Hamilton Rating Scale-Depression (she was trained by the study psychologist to do this), assessed side effects using a checklist, and reminded participants to bring the hot flash monitor with them to their week 2 visit. Week 2 was completed 7 days later. The week 2 monitor was returned by mail.

Measures

Demographic information was collected with a questionnaire (birthdates, race, marital status, education, employment status, income, current medications, menopause status, gynecological and reproductive history).

^{6,45,46} Breast cancer information was self-reported by participants and verified through medical record review (date of diagnosis, disease stage, and dates/types of treatments). In addition, participants were asked to complete the *Co-morbidity Questionnaire* to document the presence of medical problems, if the condition caused problems, required medication, and/or limited activities.⁴⁷

Safety monitoring was completed as follows. First, the *Hamilton Rating Scale-Depression*^{48,49} was used to rule out depressive symptoms at study entry, at the end of each test day, and the day after each test day (eg, score ≤ 18). This scale has been used to monitor response and/or safety in other acute tryptophan depletion studies.²⁷ Interrater reliability between the study psychologist and study nurse exceeded 90%. Second, mood and physical side effects were monitored throughout each test day (baseline and hours 3, 5, 7) and the next morning. The *Profile of Mood States-Short Form* is a 37-item scale that is based on the 65-item version.^{50,51} This list of adjectives is rated on a 0 to 4 point scale, and responses are summed to generate a total score and six subscale scores (depression, tension, anger, confusion, vigor, fatigue). This scale has been used in other studies to monitor response to acute tryptophan depletion.²⁷ Reliability and validity in women with breast cancer have been supported.⁵² The *Side Effects Report* consisted of a list of 24 physical symptoms, including symptoms previously associated with acute tryptophan depletion (nausea, vomiting, nervousness, loss of concentration).^{53,54} Respondents indicated if they were having each symptom (no, yes) and, if so, rated severity using a 0 (not at all)- to 4-point (extremely) scale. Third, *serum glucose* was assessed at each hourly blood draw (YSI Life Sciences Instrument, Yellow Springs, OH). Glucose tablets were administered by mouth for glucose values 70 mg/dL or less. All safety monitoring data were reviewed routinely by the investigative team and a data safety monitoring board.

Hot flash response was monitored as follows. *Physiological hot flash frequency* was assessed using sternal skin conductance monitoring.^{45,46,55-57} Participants wore the monitor during the test day and during the nighttime at home for a total of 24 hours. At the end of the monitoring session, the monitor was downloaded and scored using customized software and established procedures.^{45,46,55-57} Scoring was done by trained raters, with interrater reliability exceeding 90%. Sternal skin conductance monitoring is the gold standard measure of physiological or objective hot flash frequency only.^{46,58} Each hot flash is defined as an increase of 2 units within a 30-second period. *Skin conductance magnitude* was the sum total change in skin conductance with each physiological or self-reported hot flash. *Self-reported hot flash frequency, severity, and bother* were assessed using written diaries and electronic event markers during the 24-hour period. When the participants experienced a hot flash, they were instructed to push the two red buttons on the hot flash monitor, write down the time of the hot flash, and rate severity and bother in a paper diary (0 = not at all and 10 = extremely severe or 10 = extremely bothersome).

Laboratory assessments included (1) hourly measurements of circulating tryptophan, serotonin, and kynurenine during each test day and (2) assessment of tryptophan/large neutral amino acid (TRP/LNAA) ratios at the presumed 5-hour tryptophan nadir each week. Whole blood tryptophan, hydroxyl-tryptophan, serotonin, and kynurenine were assayed with high-performance liquid chromatography (HPLC) using procedures similar to that described by others.⁵⁹ The large neutral amino acids concentrations were determined by the Waters Pico-Tag methods. Briefly, the amino acids were derivatized with phenylisothiocyanate forming phenylthio-carbamyl derivatives. The resulting samples were then analyzed by reversed-phase HPLC separation and ultraviolet detections, using a Waters Alliance HPLC system.

Genetic polymorphisms in three serotonin-related candidate genes were assessed for association with the hot flash response. We chose these specific polymorphisms because previous publications have reported that they were associated with clinical phenotypes. The following single nucleotide polymorphisms (SNPs) were genotyped: rs6313 and rs799701 in the serotonin receptor 2A gene (*HTR2A*); rs1800532 from the tryptophan hydroxylase gene (*TPHI*); and rs6295 from the serotonin receptor 1A (*HTR1A*) gene. DNA was extracted from whole blood using the Centra DNA extraction kit. The *HTR1A* and *HTR2A* SNPs were genotyped using TaqMan assays from Applied Biosystems, Inc (assay identification nos. c_11904666_10 [rs6295], c_3042197_1 [rs6313], and c_1619749_10 (rs7997012)).

Genotyping for the *TPHI* SNP was conducted using an allele-specific polymerase chain reaction assay as follows: we used the iCycler system (Bio-Rad) with allele-specific primers (common forward primer: 5'-AGA ATG GTA CCT GGC ATG AAA-3', reverse primer for the allele containing A: 5'-C CTA TGC TCA GAA TAG CAG CTC T-3', reverse primer for the allele containing C: 5'-CTA TGC TCA GAA TAG CAG CTC G-3') and with SYBR Green Supermix (Bio-Rad). The allele-specific real-time polymerase chain reaction was running for 45 cycles at 95°C for 10 seconds and 55°C for 45 seconds. Alleles were discriminated based on their Ct values.

Statistical analysis

A preliminary analysis of the first four participants indicated that 25 participants were needed to provide 80% power to detect a modest and significant difference between depletion and control arms using a paired *t* test and α of 0.05.

The following analyses were done to verify experimental procedures. Sample characteristics were examined using descriptive statistics. Two-sided *t* tests and Fisher's exact tests were used to compare characteristics of participants who received depletion/control and control/depletion. To verify that adequate depletion was reached, we used (1) repeated-measures analysis to compare the percent change in serum tryptophan over time between randomized groups and (2) Fisher's exact tests to compare tryptophan values and TRP/LNAA ratios at the nadir time point (5 h after drink ingestion) between groups.

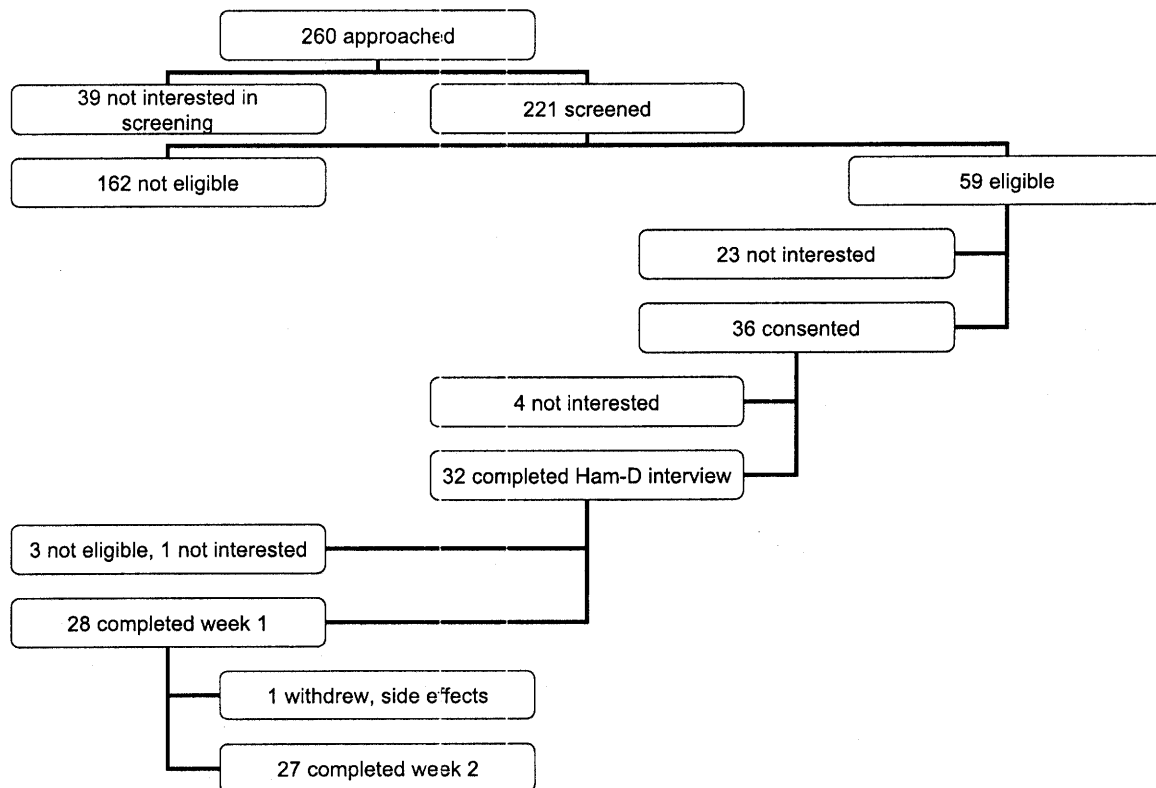


FIG. 1. Study accrual and retention: participant flow with attrition throughout the study. The Hamilton Rating Scale-Depression (Ham-D) telephone interview refers to the study psychologist verifying eligibility as nondepressed using the Ham-D (score ≤ 18).

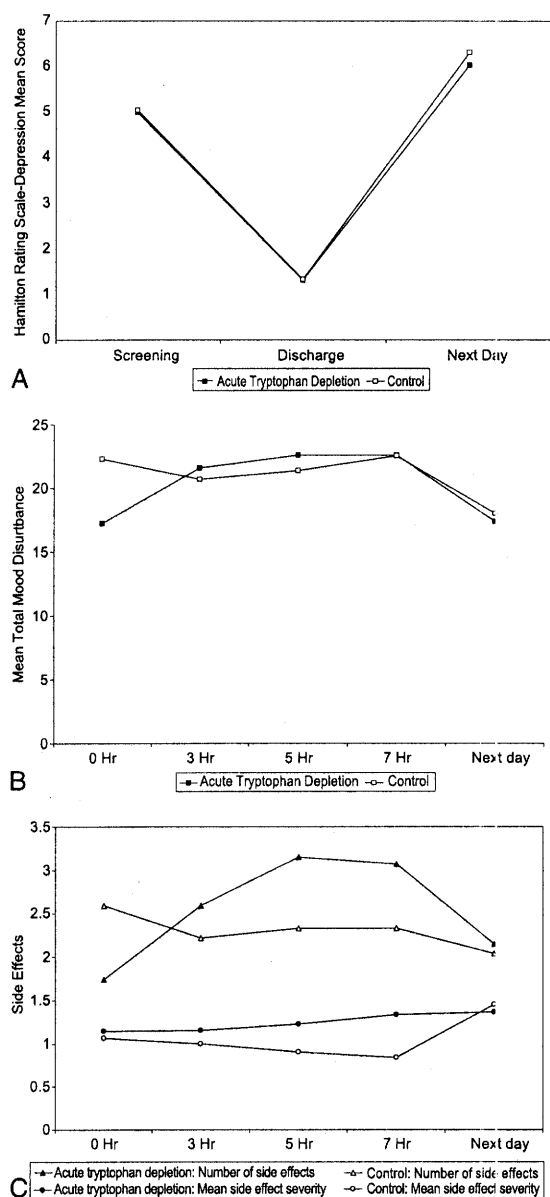


FIG. 2. Safety monitoring during acute tryptophan depletion (ATD) and control arm. **A:** The mean Hamilton Rating Scale-Depression score at each time point for the study sample ($n = 27$): initial screening to verify eligibility (as assessed by the study psychologist), at discharge from the study visit (as assessed by the study psychologist), and the following day (as assessed by a trained nurse). The solid square refers to the active amino acid drink and the open square to the control drink. Scores remained below 18 (cutoff for depression) at all time points and for both study arms. **B:** Profile of Mood States total mood disturbance scores at each time point for the study sample ($n = 27$). The solid square refers to the active amino acid drink and the open square to the control drink. Hours 0 to 7 were during the test day for each condition. Total mood disturbance scores were not significantly different over time ($P > 0.20$). **C:** Number and mean severity of side effects over time for each condition. Participants ($n = 27$) indicated if they were having symptoms using a checklist and if the number they endorsed was summed to calculate the total number of symptoms. For each symptom that they endorsed, participants rated severity from 0 (not at all) to 4 (extremely). Mean severity for all symptoms endorsed was calculated (total severity/total number of symptoms). The closed triangle and circle refer to the active amino acid drink and the open triangle and circle to the control drink. Hours 0 to 7 were during the test day for each condition. Number and severity of symptoms were not significantly different between study arms over time ($P > 0.20$).

After examining correlations among hot flash measures using Pearson's correlations, the main hypothesis was tested using two sets of hot flash variables: (1) those based on data from the total 8-hour test day (from drink ingestion to 8 h later [hours 0-8]) and (2) those based on a 3-hour tryptophan nadir period (from 5 h after drink ingestion to 3 h later [hours 5-8]). We tested both week 1 to week 2 carryover effects and week effects using a mixed linear model. Because neither of the effects were significant, we were able to ignore the order effect and collapse data for paired t tests comparing depletion to control using all participants as their own controls. Because five participants vomited at some point during the acute tryptophan depletion clinic day and that could potentially confound effects of the tryptophan depletion, we also compared hot flashes between those who achieved the greatest depletion compared with those with a less effective depletion based on (1) tryptophan concentrations less than $10 \mu\text{M}$ at hour 5 versus $10 \mu\text{M}$ or more at hour 5 and (2) TRP/LNAA less than 0.007 versus 0.007 or more. These cutoffs were determined empirically based on visual inspection of the data. For both measurements, the values appeared in one of two groups that were separated by a region of concentrations in which no samples were observed. We also tested whether the response to the acute tryptophan depletion versus control conditions varied by the following: use of SSRIs/SNRIs, antiestrogens (such as tamoxifen or aromatase inhibitors), breast cancer disease and treatment variables (time since diagnosis, type of treatment), and genetics. Because of the small sample sizes, genetic analyses were conducted by descriptive methods.

RESULTS

The accrual flow is shown in Fig. 1. Of the 36 consenting women, 5 expressed lack of interest and withdrew and 3 were withdrawn due to ineligibility. Of the remaining 28 women, 28 completed week 1 and 27 completed week 2 (1 withdrew after week 1 due to dizziness occurring 3 d after the study visit). Genetic analyses were available for all women. However, because of difficulties in sustaining venous access, only 24 women had serial tryptophan and metabolites data during the depletion week and only 23 during the control week.

The 27 participants were mostly white (93%), were married or living with a partner (82%), and had household incomes more than \$60,000 (59%). Mean (SD) age was 53.4 (9.6) years (range, 30-71 y). All had been successfully treated for nonmetastatic breast cancer (eg, stage III or less) and were considered to be free of cancer at the time of the study. Mean (SD) time after completion of primary treatment (eg, surgery, chemotherapy, and radiation) was 37.8 (18.6) months (range, 13-77 mo). Breast cancer treatments received included surgery alone (15%), surgery with radiation therapy (15%), surgery with chemotherapy (19%), and surgery with radiation and chemotherapy (52%). No participants were taking hormone therapy, 37% were taking tamoxifen, 42% were taking an aromatase inhibitor, and 33% were taking an SSRI/SNRI.

No significant differences were found between those randomized to depletion/control versus control/depletion for age, race, marital status, education, employment, income, or SSRI/SNRI use ($P > 0.10$).

Adverse events included the following. During acute tryptophan depletion, five participants vomited. Vomiting occurred within 20 minutes after ingesting amino acids ($n = 1$) or between 4 and 6.25 hours later ($n = 4$). One woman experienced hypoglycemia 3 hours after drink ingestion during both study visits that was resolved with glucose tablets. Mood was not significantly impacted. Hamilton Rating Scale-Depression scores remained less than 18 at all time points and for both study arms. Similarly, total mood disturbance and symptom rating scores were not significantly different between study arms over time ($P > 0.20$; see Fig. 2).

The percent change in serum tryptophan during depletion and control conditions is shown in Fig. 3. Baseline, 1-hour, and 2-hour total tryptophan values were not significantly different ($P = 0.46$) between the group randomized to depletion treatment first and the group randomized to depletion second. However, the tryptophan depletion group had significantly lower total tryptophan than the control group at hours 3 through 8, indicating that adequate separation between study arms was achieved. As expected, during the nadir period (5 h after drink ingestion), 83% of women receiving tryptophan depletion had total tryptophan values less than $10 \mu\text{M}$ compared with 35% in control group ($P = 0.001$). Similarly, nadir TRP/LNAA ratios were significantly lower at hour 5 during tryptophan depletion (mean [SD], 0.0034 [0.0027]) compared with control (mean [SD], 0.02 [0.02]) ($P \leq 0.0001$).

As shown in Table 1, objective hot flash frequency and magnitude were highly correlated. Similarly, subjective hot flash variables (diary frequency, event button frequency, in-

TABLE 1. Pearson's correlations denoting relationships among hot flash variables

	1	2	3	4	5	6
1. Objective frequency	—	0.96 ^a	0.68 ^b	0.71 ^b	0.69 ^b	0.64 ^b
2. Skin conductance magnitude	0.97 ^a	—	0.69 ^b	0.73 ^a	0.69 ^b	0.61 ^b
3. Subjective frequency (diary)	0.42 ^c	0.48 ^c	—	0.94 ^a	0.80 ^a	0.79 ^a
4. Subjective frequency (event button)	0.43 ^c	0.49 ^c	0.98 ^a	—	0.85 ^a	0.84 ^a
5. Subjective intensity	0.43 ^c	0.46 ^c	0.83 ^a	0.77 ^a	—	0.94 ^a
6. Subjective bother	0.32	0.35	0.77 ^a	0.74 ^a	0.89 ^a	—

The numbers in columns 2 to 6 correspond with the numbered variables listed in column 1, such that 1 is objective frequency, 2 is skin conductance magnitude, and so forth. Correlations above the diagonal are total or mean values during the entire 8-hour acute tryptophan depletion condition. Correlations below the diagonal are total or mean values during the entire 8-hour control condition.

^a $P < 0.001$.

^b $P < 0.01$.

^c $P < 0.05$.

tensity, bother) were highly correlated. Correlations between objective and subjective hot flash variables were modest ($0.46 < r < 0.58$) in this study but higher than correlations for similar data collected during ambulatory monitoring outside a laboratory ($0.06 < r < 0.26$).⁶⁰

Table 2 shows no significant differences in any hot flash variables between depletion and control conditions for any of the 8-hour long variables or any of the 3-hour long tryptophan nadir variables. Not shown are t tests for differences between groups based on tryptophan response and TRP/LNAA response. These t tests were similar and nonsignificant. In addition, response did not vary by use of SSRIs/SNRIs, antiestrogens, breast cancer disease and treatment variables, or genetic variants in the *HTR1A*, *HTR2A*, and *TRH1* genes.

DISCUSSION

The primary aim of this study was to investigate the effects of lowered serotonin synthesis by acute tryptophan depletion on hot flashes in female survivors of breast cancer—a group known to have frequent, severe, and bothersome hot flashes. It was hypothesized that acute tryptophan depletion would exacerbate hot flashes; however, our data did not support this assumption. We did not find any significant differences between study conditions for objective or subjective hot flash measures.

There are two potential explanations for these negative study findings. First, if central serotonin functioning was severely diminished in these postmenopausal women, we may not have accomplished a further reduction in serotonin with acute tryptophan depletion. Although women are generally more susceptible than men to the effects of acute tryptophan depletion on memory,⁶¹ estrogen loss at menopause may affect how postmenopausal women respond to acute tryptophan depletion. Loss of estrogen seems to diminish serotonin concentrations, activity, and metabolism.¹⁷⁻²¹ Thus, we may not have accomplished further

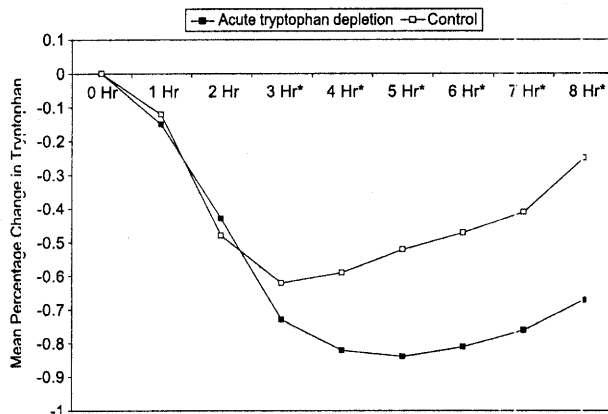


FIG. 3. Percent change in serum tryptophan from baseline over time during acute tryptophan depletion versus control arms ($n = 27$). Times 0 to 8 refer to hourly blood draws during each test day. The solid square refers to the active amino acid drink and the open square to the control drink. Repeated-measures analysis indicated that tryptophan values were not significantly different ($P = 0.46$) between randomized groups at hours 0, 1, or 2 but were significantly lower in the acute tryptophan depletion group compared with control group at hour 3 ($P < 0.01$) and hours 4, 5, 6, 7, and 8 ($P < 0.001$). Significant differences are shown with an asterisk next to the time point (ie, hours 3 through 8).

TABLE 2. Descriptive statistics for hot flash variables during acute tryptophan depletion (ATD) and control arm for 8-hour test day and 3-hour nadir periods

	8-h test day ^a			3-h nadir period ^b		
	ATD	Control	P	ATD	Control	P
Objective frequency	2.30 (2.92)	2.62 (3.29)	0.71	1.22 (1.69)	1.23 (1.66)	0.99
Skin conductance magnitude ^c	8.54 (9.17)	9.20 (11.24)	0.82	4.29 (4.92)	4.36 (5.54)	0.96
Subjective frequency (diary)	4.56 (3.11)	3.65 (3.02)	0.29	1.85 (1.66)	1.42 (1.39)	0.31
Subjective frequency (event button)	4.41 (2.93)	3.54 (3.11)	0.30	1.85 (1.49)	1.38 (1.55)	0.27
Subjective intensity	16.81 (15.87)	13.69 (16.30)	0.48	7.22 (7.83)	5.81 (7.30)	0.50
Subjective bother	14.26 (15.29)	10.23 (12.73)	0.30	6.33 (7.64)	4.65 (6.41)	0.39

Data are mean (SD).

^aFrom drink ingestion to 8 hours later.

^bThree-hour period spanning 5 to 8 hours after drink ingestion.

^cSum of all changes in skin conductance that accompanied objective and subjective hot flashes.

lowering of central serotonin despite the observed changes in peripheral tryptophan and TRP/LNAA ratios. This would need to be tested in a subsequent study using cerebrospinal fluid sampling.

If central serotonin levels are already low in these women, an alternative approach is to provide additional tryptophan by dietary supplementation. However, this assumes a substrate limitation rather than an enzyme limitation. If menopause alters the enzymes involved in converting tryptophan to serotonin, adding more substrate would not produce a response. However, there is evidence suggesting that tryptophan supplementation may have merit. For example, tryptophan supplements have been suggested as a potential hot flash treatment⁶² and seem to impact other symptoms. For example, in women, tryptophan significantly reduced premenstrual dysphoric symptoms compared with placebo.^{63,64} In humans, primates, and rats, tryptophan decreases mild depressive symptoms and aggressive behavior.^{65,66} Chronic tryptophan supplementation raises plasma tryptophan and central serotonin and potentiates SSRI effectiveness in rats.^{57,68} Although contaminated tryptophan supplements were thought to cause eosinophilia myalgia syndrome,⁶⁹ today, dietary manipulation of tryptophan or L-tryptophan/5-hydroxytryptophan supplementation is considered to be a safe intervention.^{65,66,68,70}

A second explanation for negative study findings may be that acute tryptophan depletion alone, without an additional stressor such as a thermal challenge, may not have been adequate to invoke hot flashes. A similar paradigm has been seen in panic and obsessive-compulsive disorders. Tryptophan depletion alone does not provoke panic; however, it increases susceptibility to panic so that if a stressor is applied during tryptophan depletion (ie, flumazenil), panic symptoms are exacerbated.²⁸ Similarly, symptoms of obsessive-compulsive disorder emerge after acute tryptophan depletion only when an additional stressor is present.⁷¹ In this study, tryptophan depletion may have increased susceptibility to hot flashes, but normal environmental stressors were controlled due to the laboratory setting (eg, absence of temperature and humidity fluctuations, absence of emotional stressors). This hypothesis could be tested in a follow-up study by comparing time to hot

flash onset or hot flash frequency, intensity, or bother after a stressor such as application of heating pads during acute tryptophan depletion and control conditions.

Study findings should be considered in light of study limitations. Although the sample size was comparable or larger than that seen in more than 70 similar studies included in a recent review,²⁷ the sample was limited in terms of ethnic and racial diversity, yet heterogeneous in relation to breast cancer disease and treatment characteristics. In addition, this sample of breast cancer survivors does not provide any indication of how healthy postmenopausal women who have not undergone breast cancer treatment would respond. Finally, the design was limited in that it did not include a study arm to evaluate the effects of tryptophan or 5-hydroxytryptophan supplementation. Some previous studies have used tryptophan supplementation for comparison.²⁸ However, because of reported variable increases in tryptophan and serotonin with supplementation³² and anticipated difficulties in recruiting women to take part in an additional study visit, we chose to use a one-quarter strength drink as the control condition. This control condition reduced plasma tryptophan levels more than the 25% that was expected, and the reasons for this are unknown. However, the control drink did differ from the full strength depletion in that depletion occurred to a lesser degree and with faster recovery.³²

CONCLUSIONS

In summary, to our knowledge, this was the first study to examine hot flash physiology using acute tryptophan depletion. Further testing of our study hypothesis may be warranted through tryptophan supplementation or a tryptophan depletion with stressor paradigm. Findings indicate that additional research on mechanistic pathways is needed to guide appropriate treatment in clinical practice.

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Appendix 2

Abstracts of presentations

*Carpenter, J.S. Skaar, T., Storniolo, A. M., Milata, J., Wu, J., & Yu, M. (Oct, 2008). Acute tryptophan depletion, central serotonin, and hot flashes after breast cancer. 2008 National State of the Science Congress in Nursing Research (Council for Advancement of Nursing Science), Washington, DC.

ABSTRACT

Background: Among women with breast cancer, a hot flash is a frequent, severe and bothersome symptom that is negatively related to mood, affect, and daily activities and can compromise compliance with life-saving medications (e.g., tamoxifen). Over 60% of breast cancer survivors report hot flashes, with 59% stating they are extremely severe and 44% reporting them to be extremely bothersome. Unfortunately, poor understanding of hot flash physiology limits clinicians' abilities to fully treat this symptom. Although the current non-hormonal treatment of choice for hot flashes after breast cancer targets the central serotonin system (e.g., paroxetine, venlafaxine), the role of serotonin in hot flashes has not been directly tested. Because the effectiveness of serotonergic agents has been based largely on improvement in subjective reporting of hot flashes, it is not clear whether benefits are due to physiological effects on hot flashes or due to improvements in mood or other related symptoms. In addition, these and other currently available treatments are not acceptable, appropriate, or effective for all women with breast cancer. Understanding the physiological mechanisms involved in hot flashes after breast cancer will enable us to develop more targeted behavioral and/or pharmacological therapies to be used in lieu of, or in addition to, currently available therapies to eradicate hot flashes and improve the quality of life for women with breast cancer.

Objective/Hypothesis: The study purpose was understand the role of serotonin in hot flashes by altering central serotonin concentrations using a well-established acute tryptophan depletion paradigm. The main hypotheses were (1) alterations in central serotonin levels are involved in the induction of hot flashes in women with breast cancer and (2) variability in response to serotonin manipulation can be partly explained by genetic variations in serotonin receptors and transporters.

Study Design: A within subjects, double blind, placebo controlled, balanced, crossover study design was used. After fasting overnight, each participant took part in two similar 9-hour test days within the General Clinical Research Center. On one test day, women with breast cancer ingested a concentrated amino acid drink and encapsulated amino acids (no tryptophan) according to published procedures that have been previously shown to have specific effects on serotonin within 4.5 to 7 hours. On the other test day, women ingested a ¼ strength amino acid drink that was identical in taste, color and volume and was previously shown to have no effects on serotonin. Women were assessed the day after each test day for adverse effects. Serial venous blood sampling was used throughout each test day to monitor response to each condition. Hot flashes were monitored using objective sternal skin conductance monitoring.

Relevance: Results will help guide development of improved interventions for alleviating hot flashes in women with breast cancer. If findings are positive, future interventions may target the central serotonin system either behaviorally (e.g., diet) or pharmacologically (e.g., alternative drug therapeutics). If findings are negative, they will be equally as useful in guiding future research on non-serotonin related etiologies and interventions.

- Carpenter, J. S., Skaar, T., Storniolo, A. M., Jin, Y., Desta, Z., & Johns, S. (Feb, 2007). The role of serotonin in hot flashes after breast cancer. 9th National Conference on Cancer Nursing Research, Hollywood, CA.

Title

THE ROLE OF SEROTONIN IN HOT FLASHES AFTER BREAST CANCER

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Abstract content (limit to 350 words)

Significance: Although hot flashes are frequent, severe and bothersome for women with breast cancer, a lack of understanding of hot flash physiology undermines successful treatment. Although selective serotonin receptor inhibitors are the treatment of choice for hot flashes after breast cancer, the role of serotonin in hot flashes has not been directly tested. Understanding serotonin's role in hot flashes will enable development of more targeted behavioral and/or pharmacological therapies.

Problem/Purpose: Purposes are to (1) directly manipulate the central serotonin system and evaluate effect on hot flashes and (2) evaluate genetic variations in serotonin receptors and transporters that may predict response to serotonin manipulation.

Framework: Our framework is based on the acute tryptophan depletion paradigm, physiological models of hot flash etiology and published literature on the role of the tryptophan-degrading enzyme, indoleamine 2,3-dioxygenase, in breast cancer.

Methods & analysis: Using a within subjects, double blind, placebo controlled, balanced, crossover study, each participant takes part in two 9-hour test days. One day, participants ingest a concentrated amino acid drink and encapsulated amino acids (no tryptophan) with specific effects on serotonin within 4.5 to 7 hours. On the other day, women ingest a control drink with no effects on serotonin. Participants arrive fasting, provide serial blood samples, take part in side effects assessments, and wear a hot flash monitor. Accrual of study participants will continue through 2007. Preliminary results will be presented.

Findings and implications: Results will guide development of improved or novel interventions for alleviating hot flashes in women with breast cancer. If serotonin is found to play a role in hot flashes, interventions may be developed to target the central serotonin system behaviorally (e.g., diet) or pharmacologically (e.g., novel drugs). If serotonin manipulation does not affect hot flashes, findings will guide future research on non-serotonin related etiologies and interventions.

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The Role of Serotonin in Hot Flashes after Breast Cancer

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Introduction: Although hot flashes are frequent, severe and bothersome for women with breast cancer, a lack of understanding of hot flash physiology undermines successful treatment. Although selective serotonin receptor inhibitors are the treatment of choice for hot flashes after breast cancer, the role of serotonin in hot flashes has not been directly tested. Understanding the role of serotonin in hot flashes will enable development of more targeted behavioral and/or pharmacological therapies to be used in lieu of, or in addition to, currently available therapies to meet our ultimate goal of preventing or eradicating hot flashes to improve quality of life.

Methods: We are altering central serotonin concentrations using a acute tryptophan depletion paradigm and are also evaluating genetic variations in serotonin receptors and transporters that may predict response to the depletion. Using a within subjects, double blind, placebo controlled, balanced, crossover study, each participant takes part in two similar 9-hour test days. On one test day, participants ingest a concentrated amino acid drink and encapsulated amino acids (no tryptophan) according to published procedures that have been shown to have specific effects on serotonin within 4.5 to 7 hours. On the other test day, women ingest a ¼ strength amino acid drink that is identical in taste, color and volume that has been previously shown to have no effects on serotonin. Women fast for 8 hours prior to each test day and will be assessed the day after each test day for adverse effects. Serial venous blood sampling is used to monitor response to each condition. Hot flashes are measured using objective sternal skin conductance monitoring.

Results: Three of four participants have had fewer hot flashes with acute tryptophan depletion (8 hot flashes total) compared to the control condition (16 hot flashes total). The fourth participant had 7 hot flashes with tryptophan depletion and 4 with the control condition. Accrual of study participants continues with additional data to be presented.

Conclusions: Results from this study will guide the development of improved or novel interventions for alleviating hot flashes in women with breast cancer. If serotonin is found to play a role in hot flashes, interventions may be developed to target the central serotonin system behaviorally (e.g., diet) or pharmacologically (e.g., novel drugs). If direct manipulation of the serotonin system does not affect hot flashes, these findings will be equally as useful in guiding future research on non-serotonin related etiologies and interventions.

- Carpenter, J., Johns, S., Storniolo, A.M., Jin, Y., Desta, Z. & Skaar T. (Feb, 2006). Update on the Role of Serotonin in Hot Flashes after Breast Cancer. The Amelia Project: Giving Wings to Research, Indianapolis, IN.

The Role of Serotonin in Hot Flashes after Breast Cancer

*Carpenter, J., Johns, S., Storniolo, A.M., Jin, Y., Desta, Z. & Skaar T.
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Introduction: Although hot flashes are a frequent, severe and bothersome symptom for women with breast cancer, limitations in our understanding of hot flash physiology undermine successful prevention or treatment of hot flashes. Although the current non-hormonal treatment of choice for hot flashes after breast cancer targets the central serotonin system (e.g., paroxetine, venlafaxine), the role of serotonin in hot flashes has not been directly tested. Understanding the physiological mechanisms involved in hot flashes after breast cancer, including the role of serotonin, will enable us to develop more targeted behavioral and/or pharmacological therapies to be used in lieu of, or in addition to, currently available therapies so that we can prevent or eradicate hot flashes and improve the quality of life for women with breast cancer.

Methods: We are altering central serotonin concentrations using a well-established acute tryptophan depletion paradigm and are also evaluating genetic variations in serotonin receptors and transporters that may predict response to the depletion. Using a within subjects, double blind, placebo controlled, balanced, crossover study, each participant takes part in two similar 9-hour test days within the General Clinical Research Center. On one test day, women with breast cancer ingest a concentrated amino acid drink and encapsulated amino acids (no tryptophan) according to published procedures that have been previously shown to have specific effects on serotonin within 4.5 to 7 hours. On the other test day, women ingest a ¼ strength amino acid drink that is identical in taste, color and volume that has been previously shown to have no effects on serotonin. Women fast for 8 hours prior to each test day and will be assessed the day after each test day for adverse effects. Serial venous blood sampling throughout each test day is used to monitor response to each condition. Hot flashes are monitored using objective sternal skin conductance monitoring.

Results: The study is funded and has accrued two participants to date. The first participant, an SSRI user, had 14 objective hot flashes during the control condition and complete relief (0 hot flashes) during the tryptophan depletion (the opposite response of what had been hypothesized). The second participant experienced 10 objective hot flashes during the tryptophan depletion and 5 during the control condition. Accrual of study participants continues with additional data to be presented.

Conclusions: Results from this study will guide the development of improved or novel interventions for alleviating hot flashes in women with breast cancer. If serotonin is found to play a role in hot flashes, interventions may be developed to target the central serotonin system behaviorally (e.g., diet) or pharmacologically (e.g., novel drugs). If direct manipulation of the serotonin system does not affect hot flashes, these findings will be equally as useful in guiding future research on non-serotonin related etiologies and interventions.

#**Carpenter, J., Johns, S., Monahan, P., Storniolo, A.M., Jin, Y., Desta, Z. & Skaar T.** (Feb, 2005). The Role of Serotonin in Hot Flashes after Breast Cancer. Poster presented at the annual meeting of The Amelia Project: Giving Wings to Research, Indianapolis, IN.

The Role of Serotonin in Hot Flashes after Breast Cancer

Carpenter, J., Johns, S., Monahan, P., Storniolo, A.M., Jin, Y., Desta, Z. & *Skaar T.

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Background: Among women with breast cancer, hot flashes are a frequent, severe and bothersome symptom. For this group, hot flashes are negatively related to mood, affect, and daily activities and can compromise compliance with life-saving medications (e.g., tamoxifen). Over 60% of breast cancer survivors report hot flashes, with 59% stating they are extremely severe and 44% reporting them to be extremely bothersome. Unfortunately, limitations in our understanding of hot flash physiology limit clinicians' abilities to fully treat this symptom. Although the current non-hormonal treatment of choice for hot flashes after breast cancer targets the central serotonin system (e.g., paroxetine, venlafaxine), the role of serotonin in hot flashes has not been directly tested. Because the effectiveness of these agents has been based largely on improvement in subjective reporting of hot flashes, it is not clear whether benefits are due to physiological effects on hot flashes or due to improvements in mood or other related symptoms. In addition, these and other currently available treatments are not acceptable, appropriate, or effective for all women with breast cancer. Our recent laboratory experiments have shown that breast cancer tissues actively metabolize tryptophan to the kynurenine metabolite. Since reduced circulating tryptophan levels can limit central serotonin synthesis, the tryptophan metabolism in breast cancer tissue may contribute to reduced quality of life and increased susceptibility to side effects of cancer therapies, such as hot flashes. Understanding the physiological mechanisms involved in hot flashes after breast cancer will enable us to develop more targeted behavioral and/or pharmacological therapies to be used in lieu of, or in addition to, currently available therapies so that we can eradicate hot flashes and improve the quality of life for women with breast cancer.

Objective/Hypothesis: The *purpose of this proposal* is to improve our understanding of the role of serotonin in hot flashes by altering central serotonin concentrations using a well-established acute tryptophan depletion paradigm. The *main hypothesis* is that alterations in central serotonin levels are involved in the induction of hot flashes in women with breast cancer and that variability in response to serotonin manipulation can be partly explained by genetic variations in the serotonin receptors and transporters.

Study Design: A within subjects, double blind, placebo controlled, balanced, crossover study design is proposed. Each participant will take part in two similar 9-hour test days within the General Clinical Research Center. On one test day, women with breast cancer will ingest a concentrated amino acid drink and encapsulated amino acids (no tryptophan) according to published procedures that have been previously shown to have specific effects on serotonin within 4.5 to 7 hours. On the other test day, women will ingest a ¼ strength amino acid drink that is identical in taste, color and volume that has been previously shown to have no effects on serotonin. Women will fast for 8 hours prior to each test day and will be assessed the day after each test day for adverse effects. Serial venous blood sampling will be used throughout each test day to monitor response to each condition. Hot flashes will be monitored using objective sternal skin conductance monitoring throughout each test day and through to the day following each test day to monitor for any longer-term adverse effects.

Translational significance: This study has recently been funded and accrual is now beginning. Results from this study implicating a direct effect of central serotonin on objective hot flashes will help guide the development of improved interventions for alleviating hot flashes in women with breast cancer. These interventions may target the central serotonin system either behaviorally (e.g., diet) or pharmacologically (e.g., alternative drug therapeutics). If direct manipulation of the serotonin system does not affect hot flashes, these findings will be equally as useful in guiding future research on non-serotonin related etiologies and interventions. Findings

from this study will ultimately be used to eradicate hot flashes as a frequent, severe and bothersome breast cancer treatment related condition, thereby, improving quality of life for all women with breast cancer.

*Von Ah, D., Skaar, R., Unverzagt, F., Yu, M., Wu, J., Moser, L., Ryker, K., Milata, J., & Carpenter, J.S. (Feb, 2009). Cognitive dysfunction in breast cancer survivors: The role of serotonin. 10th National Conference on Cancer Nursing Research, Orlando, Florida.

Abstract

SIGNIFICANCE: Although cognitive dysfunction is a prevalent and disruptive problem for many breast cancer survivors (BCS), little research has examined its etiology. One potential mechanism that remains to be explored is serotonin. Serotonin receptors are located in brain areas implicated in normal and dysfunctional cognitive processes and serotonin levels are significantly affected by estrogen withdrawal—a common side effect of breast cancer treatment. However, no study has evaluated serotonin's impact on cognitive dysfunction in BCS.

PURPOSE: The purpose of this study was to examine the role of serotonin in cognitive dysfunction in BCS by lowering central serotonin concentrations via acute tryptophan depletion. We hypothesized that alterations in central serotonin levels induce cognitive dysfunction in women with breast cancer.

SCIENTIFIC FRAMEWORK: The acute tryptophan depletion paradigm was used.

METHODS: A within-subjects, double blind, placebo controlled, crossover study was used. 20 female BCS who were 30-66 years old ($M = 51.2$, $SD = 9.3$) and on average 32.8 months post-treatment (range 13 – 57 months) for non-metastatic breast cancer were recruited. Consenting participants received (a) acute tryptophan depletion according to published procedures that have been shown to reduce central serotonin levels or (b) a control condition in random order during two test days at the General Clinical Research Center. Cognitive dysfunction was measured at the same time each test day using standardized neuropsychological tests to evaluate short and long-term memory recall; attention and concentration; executive function and language; cognitive processing speed; and psychomotor ability. Data were analyzed using descriptive statistics, MANOVA for overall differences between groups and MANCOVA to adjust for potential covariates (e.g., age, education).

RESULTS: BCS performed significantly worse on a long-term memory recall test and on a psychomotor ability test when serotonin was lowest during tryptophan depletion versus control ($p < .01$ -.05).

IMPLICATIONS: Findings suggest that reductions in serotonin may specifically impact long-term memory recall and psychomotor ability in BCS. Although further investigation is warranted these findings have the potential to help guide the development of interventions targeting the central serotonin system either behaviorally (e.g., diet) or pharmacologically. Findings will ultimately be used to reduce cognitive dysfunction in BCS.

Key words: cognitive dysfunction, serotonin, and breast cancer

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*Von Ah, D., Carpenter, J. S., Unverzagt, F., & Skaar, T. (Mar, 2008). "Cognitive Dysfunction in Breast Cancer Survivors: The Role of Serotonin," 2008 Annual Midwest Nursing Research Conference, Indianapolis, IN.

Abstract

SIGNIFICANCE OF THE STUDY: Although cognitive dysfunction is a prevalent, persistent, and disruptive problem for many breast cancer survivors, little research has examined its etiology.

PURPOSE: We are examining the role of serotonin in cognitive dysfunction. Hypotheses are (1) alterations in central serotonin levels induce cognitive dysfunction in women with breast cancer and (2) variability in response to serotonin manipulation can be partly explained by genetic variations in serotonin receptors and transporters.

SCIENTIFIC FRAMEWORK: A biobehavioral model using the acute tryptophan depletion paradigm is being used.

METHODS & ANALYSIS: This is a within-subjects, double blind, placebo controlled, crossover study. 30 female breast cancer survivors who are >1 month but < 5 years post-treatment (surgery, radiation, chemotherapy) for non-metastatic breast cancer will be recruited from a cancer center. Consenting participants will receive acute tryptophan depletion based on published procedures or a control condition in random order during two test days at the General Clinical Research Center. On one day, participants will ingest a concentrated amino acid drink and encapsulated amino acids (no tryptophan) according to published procedures that have been shown to have specific effects on serotonin within 4.5 to 7 hours. On the other day, women will ingest a control drink with no effects on serotonin. Serial venous blood sampling is used to monitor response to each condition and to investigate genetic polymorphisms that may affect response. Cognitive dysfunction will be measured at the same time each test day using standardized neuropsychological tests. Data will be analyzed using descriptive statistics, MANOVA for overall differences between groups and MANCOVA to adjust for potential covariates such as age, education, previous medical treatment and current medications. Our main hypothesis will be supported if cognitive dysfunction is exacerbated during acute tryptophan depletion.

FINDINGS AND IMPLICATIONS: Results will help guide the development of improved or novel interventions targeting the central serotonin system either behaviorally (e.g., diet) or pharmacologically. Null results will be equally as useful in guiding future research on non-serotonergic etiologies and interventions. Findings will ultimately be used to reduce cognitive dysfunction and improve quality of life breast cancer survivors.

*Von Ah, D., Carpenter, J. S., Unverzagt, F., & Skaar, T. (Feb, 2008). Cognitive dysfunction in breast cancer survivors: The role of serotonin. 10th Annual Amelia Retreat, IU Conference Center, Indianapolis, IN.

Abstract

INTRODUCTION: Although cognitive dysfunction is a prevalent, persistent, and potentially debilitating problem for the growing number of breast cancer survivors, little research has examined its etiology. We are examining the role of serotonin in cognitive dysfunction. Hypotheses are (1) alterations in central serotonin levels induce cognitive dysfunction in women with breast cancer and (2) variability in response to serotonin manipulation can be partly explained by genetic variations in serotonin receptors and transporters. A biobehavioral model using the acute tryptophan depletion paradigm is being used.

METHODS: This is a within-subjects, double blind, placebo controlled, crossover study. 22 female breast cancer survivors who are > 1 month but < 5 years post-treatment (surgery, radiation, chemotherapy) for non-metastatic breast cancer have been recruited from the IU Melvin and Bren Simon Cancer Center. Consenting participants receive acute tryptophan depletion based on published procedures or a control condition in random order during two test days at the General Clinical Research Center. On one day, participants will ingest a concentrated amino acid drink and encapsulated amino acids (no tryptophan) according to published procedures that have been shown to have specific effects on serotonin within 4.5 to 7 hours. On the other day, women will ingest a control drink with no effects on serotonin. Serial venous blood sampling is used to monitor response to each condition and to investigate genetic polymorphisms that may affect response. Cognitive dysfunction will be measured at the same time each test day using standardized neuropsychological tests. Data were analyzed using descriptive statistics, t-tests, and MANOVA for overall differences between groups. MANCOVA to adjust for potential covariates such as age, education, previous medical treatment and current medications will be performed. Our main hypothesis will be supported if cognitive dysfunction is exacerbated during acute tryptophan depletion.

RESULTS: 22 women consented to participate in the study, with 20 having usable data for analyses. The women ranged in age from 30-66 years old, with mean age of 51.2 (SD=9.3), with the majority Caucasian (90%), married (65%), and a household income > \$60,000 (65%). Medical and treatment information revealed that the women were on average 32.8 months post treatment (SD=14.3; range 13-57 months) with Stage II disease or less (75%). Most received a lumpectomy (50%), chemotherapy (75%), and/or radiation (60%). 75% were currently receiving anti-estrogen therapy such as tamoxifen or an aromatase inhibitor. Women reported overall poor memory performance. **Preliminary results of the objective neuropsychological tests revealed a significant treatment effect with women receiving tryptophan depletion performing significantly worse on the Rey AVLT delayed recall test and finger tapping test than when receiving control.** These findings suggest reductions in tryptophan/serotonin may impact long-term memory performance and psychomotor ability of breast cancer survivors.

CONCLUSIONS: Data analyses are continuing. Laboratory analyses of genetic variations in serotonin receptors and transporters will be conducted once assays are completed. Final results of this study will help guide the development of improved or novel interventions targeting the central serotonin system either behaviorally (e.g., diet) or pharmacologically. Null results will be equally as useful in guiding future research on non-serotonergic etiologies and interventions. Findings will ultimately be used to reduce cognitive dysfunction and improve quality of life breast cancer survivors

*Von Ah, D., Carpenter, J. S., Skaar, T. (Feb, 2007). Serotonin and cognitive dysfunction in breast cancer survivors. National Conference on Cancer Nursing Research, Hollywood, CA.

Abstract

SIGNIFICANCE OF THE STUDY: Although cognitive dysfunction is a prevalent, persistent, and disruptive problem for many breast cancer survivors, little research has examined its etiology.

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FINDINGS AND IMPLICATIONS: Results will help guide the development of improved or novel interventions targeting the central serotonin system either behaviorally (e.g., diet) or pharmacologically. Null results will be equally as useful in guiding future research on non-serotonergic etiologies and interventions. Findings will ultimately be used to reduce cognitive dysfunction and improve quality of life breast cancer survivors.